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Research Article

Lipid Extraction and Optimization for Biodiesel Production from Blended Waste Water Treatment Sludge Disposal with Algae

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Abstract

Waste sewage sludge is the most pollutant that affects our environment which constituent's major organic volatile materials and it represents lipid components. And also non-renewable energy engine consumption releases high amounts of oxide pollutants to the environment; in addition the depletion non-renewable energy like petroleum, natural gas fossil fuel increases from time to time over the world with the demand increment. In the recent time production of biodiesel established mostly from edible oil for the coverage of non-renewable energy depilation. But also it again became great challenges on food security. Therefore seeking another opportunity that lipid extracting from algae and sludge is the recent studies for substitution of consumable and non-renewable raw materials and farther treatment for sewage sludge wastes. The objective of this study was extraction and optimization of lipid from blended sludge disposal with algae for biodiesel production. The procedures are fist characterize algal and sludge wastes, extract lipid component by Soxhlet extraction. From the experiment the optimization was achieved at 80°C and 6 h retention time, that 47% lipid recovered from 20 g equal dried blended sludge and algae using 200 ml n-hexane solvent. And also design model equation where developed using design expert software with in the factor ranges (time 3 to 6 h and temperature 75 to 85°C).

Keywords: Lipid; Extraction; Solvent; Sludge algae; Temperature; Optimization.

Introduction

Sewage sludge is a residual material left behind from the treatment of municipal wastewater [1]. That includes, household waste liquid from toilets, baths, showers, kitchens, sinks and so forth that is disposed of via sewers and industrial waste water treatment sludge disposal, Sewage sludge is a complex heterogeneous mixture of microorganisms, undigested organics such as paper, plant residues, oils and faecal material, and moisture [2]. Sewage sludge has been used as a fertilizer in many areas all over the world [3]. In the recent time sewage sludge become the source of energy generation in advanced technology. It can also contribute in solving a number of problems of energy supply and healthy advanced of environment and being a renewable energy source [4].

Algae are organisms that grow in aquatic environments and use light and carbon dioxide (CO_2) to develop their structural cell [5]. Multi cellular algae mostly growth & survive in every oxidation ponds around warm and humid areas [6].

Fig. 1 shows microalgae grow and survive in the oxidation pond that more at the end process of maturation pond section. At the same time, at the end of the maturation pond algae die because of enough old and with unfavourable condition happen [7]. Died algae settled down and become sludge which contains high concentrated of the lipid [8]. Oil content in microalgae can exceed 70% dry weight of biomass [9].

In recent time, the interest sustainable energy production increased as the consideration that they can be a suitable source of third generation bio fuels such as crude oil (triacylglycerols) or after conversion to methyl esters (biodiesel) [10]. Composition of lipids in algal greatly depends on genetic and phenotypic factors, including environmental and culture conditions [11]. Under unfavourable environmental or stress conditions many microalgae alter their lipid biosynthetic

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pathways towards the formation and accumulation of neutral lipids (20–50% dry cell weight), mainly in the form of Triacylglyceride (TAG), enabling microalgae to endure these adverse conditions [12].

Fig. 1. Microalgae survival areas

Biodiesel is one of the most promising renewable fuels proposed as an alternative to fossil diesel [13]. Biodiesel is predominantly produced from vegetable edible oils; more than 95% of the world's biodiesel is produced from edible vegetable oils [14]. However, the competitive potential of biodiesel is currently limited by the high price of the common lipid feed stocks [15], which constitutes between 70-85% of the overall biodiesel production cost, strongly influencing the final price of this bio fuel. In addition, the excessive cultivation of edible oil seeds for biodiesel raises the concerns of food shortage, which competes with fuel production [16]. Therefore, a low-cost and non-edible feedstock is required in order to reduce the production cost and to facilitate competitiveness with petroleum diesel [17].

This research investigates the utilization of municipal wastewater sludge blended with microalgae as a source of lipid feedstock for the production of biodiesel. Waste water treatment sludge disposal and algae are the most available in large quantities and non-edible feedstock, which can make the biodiesel production profitable [18]. Because waste water treatment sludge disposal is a source of lipid and the average organic matter content of sewage sludge in China is 38.4%; of which, 55% are carbohydrates, 20% are proteins, approximately 20% are lipid [19]. All activated sludge contained an abundance of cellular lipids (>54%); Cells in sludge can from plants, animals, microbes and so on in wastewater [19]. Therefore the waste sewage sludge recovery and use for lipid extraction is advanced technology for sustainable energy development in green environment. Lipids are often defined by low solubility in water and high solubility in non-polar solvents, such as hexane, chloroform and diethyl ether. Lipids are often classified into two groups; compounds with polar heads and a long non polar tail and fused-ring compounds [20]. The first group includes triglycerides, fatty acids and phospholipids among others and the second group includes cholesterol as one of the most important fused-ring compounds [21].

The requirement of lipid extraction and biodiesel production process: Soxlet extraction is a technique used to extract organic material from a solid. By refluxing the solvent the solid is washed repeatedly and extracted material drops down and is collected in a flask. Transisterification is when one ester is converted to another ester. Triglycerides can undergo transisterification since they are already esters [22]. Transisterification is a method that chemically converts oils/fats to its corresponding fatty ester. The conversion is done by exchanging an organic group R1 of an ester with the organic group R2 of an alcohol in the presence of an acid or a base as catalyst [23].

The mechanism for synthetization of esters by acid-catalyzed transisterification is similar to that for an acid-catalyzed esterification, but water is not formed. Triglycerides are converted to diglyceride followed by monoglyceride and at last glycerol. For each step a molecule of a methyl ester of fatty acid is produced [24].

Triglyceride + ROH ↔ Diglyceride + RCOOR1
Diglyceride+ROH↔ Monoglyceride + RCOOR2
Monoglyceride + ROH ↔ Glycerol + RCOOR3

Energy has become a crucial factor for humanity. Nowadays, modern society consumes large amounts of energy to maintain a high standard of living and to ensure economic growth and development [25]. Currently 87% of all energy consumed worldwide is from fossil fuel sources [26]. Oil contamination can affect soil physical and chemical properties, the daily maximum surface temperature of hydrocarbon contaminated soil are often higher than that of adjacent control sites [27]. Fig. 2 shows the algae decomposed in waste water, fresh and developed algae.

Fig. 2. Sludge after waste water treatment and algae

Lipid from wastewater sludge is originated from algae. Therefore besides that suitable lipid fraction, sewage sludge is considered as potential lipid feedstock for biodiesel production due to its advantages over the conventional vegetable oils such as:

Sewage sludge is non-edible lipid feedstock; therefore the competition with the food market is eliminated. Sewage sludge is cheap or practically costless feedstock, as it is a waste generated in WWTPs, therefore the cost of biomass production and land requirement is eliminated. Sewage sludge is readily available and in large quantities as the waste is steadily generated in abundance in WWTPs. Furthermore, the use of sludge as a source of lipid for biodiesel production is also an alternative to exploit the excess of waste sludge, consequently lowering WWTP operation cost [28].

Extract and optimization of Lipid from blended sewage sludge and algae for biodiesel production and extraction optimisation model using design expert software is the major objective of the present work. Specific objectives are to identify and characterizing of the blended of municipal sewage sludge wastes and microalgae, extract the lipid components using soxhlet extraction process and optimise the extraction model using design expert software

Methodology

The study method where covered with experimental laboratory and software programming.

Chemical requirements

Hexane (purity >97%), Sulphuric acid (purity >99.999%), Methyl heptadecanoate (purity>99.0%), Sodium chloride, Sodium hydrogen carbonate

Materials requirements

Soxhlet for extraction apparatus, pH meter, Sludge collector, Furnace, Liquid chromatography, Heating mantle - explosion-proof, with temperature control, Boiling flask – 125 mL or appropriate size, Analytical balance - capable of weighing, Vacuum pump, or other vacuum source, Paper extraction thimble for Soxhlet apparatus, Glass wool or small glass beads to fill thimble.

Lipid extraction for biodiesel production process

The followings are the steps followed, collection of municipal sewage sludge and Algae from oxidation ponds of Addis Ababa and Jimma. characterizing of blended sample sewage sludge algae, using proximate analysis, extract the lipid component using soxhlet apparatus, analysis quality of lipid extracted, optimize and model using design expert software, and convert the lipid in to biodiesel using transisterification process

After sample was collected, proximate analysis was done: by determining total solid, fixed solid, volatile solid, moisture content, ash content and all other relative properties by proximate analysis [29]. The next process was extraction of the components of the lipid from blended municipal sewage sludge and algae using soxlet extraction apparatus with the chemical of hexane [30].

In the fig. 3 there are three sections: the first is extraction section which lipid components extracted using solvent extracts, the second section shows the evaporation or separation section that separates hexane solvent from solute lipid, the last section is reaction or transisterification reaction that the section which converts glycerol (lipid) in to ester.

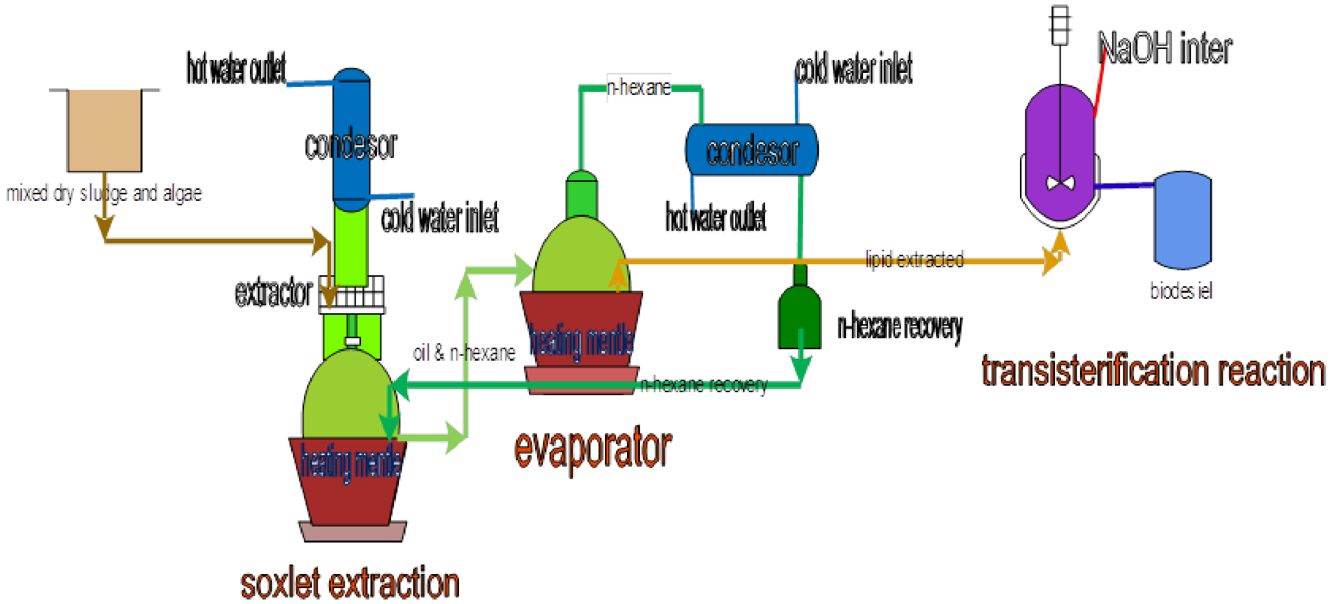


Fig. 3. Overall lipid extraction and biodiesel production from waste for sustainable development

Raw material sample preparation

Sludge samples were collected from one of the primary waste water treatment bedele brewery waste water treatment. Sludge samples (10 L) which moisture content was 90% taken to jimma institute of technology, which is about 70 km far from bedele brewery industry. Algae sample (10 L) collected from the Jimma institute of technology oxidation pond and Jimma boye rever which moisture content was 95%. After drying the sludge and algae in sun for three, the moisture contents for both approximately became about 20%. The samples where characterized after drying in sun for three days proximate analysis.

Result and discussion

Raw material characterization

Physical characterization

Moisture content (M): moisture content determined by using oven dryer, first the sample was weighted second after drying at 108±2℃ for 1.5 hr [31]. Equation (1) is used to determine the moisture contents by subtracting final dried weight of sample from initial dried weight of sample.

$$M = Mi - Mf \qquad (1)$$

Volatile Matter (V): volatile matter determined using muffle furnace. First dry sample was weighted the next weighted the sample after heated in the furnace for 7 min at 900± 10°C [32]. Equation (2) is similar with the equation 1 but the only difference is temperature and time.

$$V = W_1 - W_2 \qquad (2)$$

Ash content: also determined using furnace, first dry sample was weighted and weighted after the sample heated at 815 ± 10°C for 45 min [32]. Equation (3) is used to determine the ash content of sample using furnace after removing moisture content and volatile mater.

$$A = g_1 - g_2 \qquad (3)$$

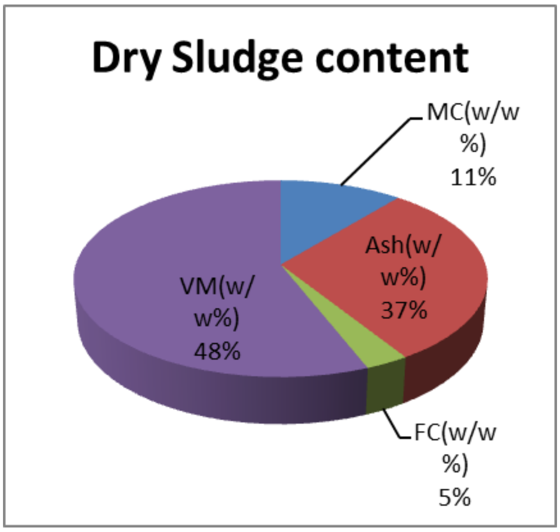
Fixed carbon: Equation (4) shows, how FC is determine from the original weight samples, the remaining sample component after subtracting moisture content, volatile mater and ash content from the original sample. When 100% weight of original sample is used.

$$FC = 100 - (M - V - A) \qquad (4)$$

Wastewater sludge and dry algae were characterized in proximate analysis and differentiated in moisture content, ash content, fixed carbon and volatile mater shown in table 1. From fig. 4 volatile mater in sludge is lower than in dry algae, so this concluded that dry algae lipid content is higher, it is the source of lipid. From the result of sample proximate analysis volatile matter was the highest component compared with other contents [33]. High component volatile matter in the sample shows high oil content. Ash content was the highest component next to volatile matter which shows [34].

Table 1. Summary of raw material characteristics

Measurement (%,w/w)	Sludge	Algae
M	10	11
ASH	37	30
FC	5	3
VM	48	56



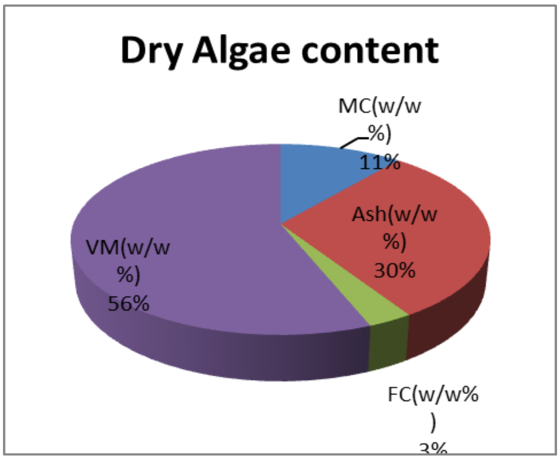


Fig. 4. Characteristics of sludge and algae by proximate analysis

Experimental procedure and results of lipid extraction

Equal proportional of dried sludge and algae blended together. An empty thimble and an empty round bottom flask were weighed. After adding up the dried blended sample (20 g) into the thimble, it was weighed again and the reading was recorded. Then, 200 mL of hexane was added into 250 mL round bottom flask. The heating process was monitored to allow 80 cycles of the extraction in approximately 4 – 6 hr. The hexane was removed from the flask by rotary evaporator after the lipid had been extracted. Then, the lipids were stored in desiccators and weighed the next run. The yield of Extracted material was determined gravimetrically and expressed as g of extractable lipids per g of dry sludge. The step was repeated by changes factors (time of extraction and temperature).

Experimental result

The lipid content was calculated as the total crude lipid yield for each experimental set. The extracted sample was calculated as percentage based by the equation (5). Equation (5) is technical oil determination equation though soxhlet extraction process unit operation. This equation is scientific and practically visible.

Oil Extraction (%)=(w2 - w1)/(m_2 - m_1)×100 (5)

Where m1 represents the mass (g) of thimble, m2 is the total mass (g) of thimble and sample (g), w1 is the mass (g) of round bottom flask, and w2 is the total mass of round bottom and lipid after extraction (g). Table 2 shows an alternative factor selections for solution out put of heist oil yeilds in the diffirent experimental run.

From the experimental result it shows that there were diffirent results from parameter change that changes the product (yield of oil). Depending on the factors that changes the product thirteen iteration of experiment where done. From the iteration (run) at eleventh, the result (oil yeild) where maximum. Therefore according to the experiments at the temperature of eighty with the retention time of six hours the yeild were fourty seven which was maximum. In the fig. 5 shows that relative factor enfluence the result or oil yeilds, In general for the blended sample sludge disposal and algae proximate characterized fourty seven percent of oil where extracted from the 54% oil content sludge and 70% oil dry biomass of algae sited in literature.

Fig. 6a and Fig. 6b are temperature vs oil yeild and time vs oil yeild graph respectively on the yeild of oil extraction results, the oil yeild and temperature graph shows, the effect of temperature on the yeild at constant time five hours. Then after 85°C there was no more changes of oil extraction. In the the next oil yeild and time graph shows the effect of time on the yeild of oil extraction at constant temperature 80°C. Therefore there were no more change of oil extraction after 6 hr.

Fitting Model Development from Experimental Result, Using Design Expert

The table 3 is alternative "Sequential Model Sum of Squares": Select the highest order polynomial where the additional terms are significant and the model is not aliased. The cubic model is selected. Want the selected model to have insignificant lack-of-fit, it suggested that linear lack of fit test as given in table 4. Focus on the model maximizing the Adjusted R-Squared and the "Predicted R-Squared are given in table 5.

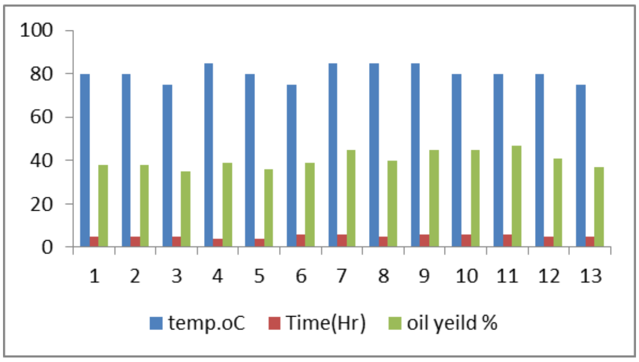


Fig. 5. Bar chart with factor enfluences the yeilds of oil extraction

Table 2. Summary of product recovery in each experimental runs

Run	Factor1 Temp(°C)	Factor 3 Time (hr)	W1(g)	W2(g)	M1(g)	M2(g)	Oil (%)
1	80	5	200	152.50	60	58.75	38
2	80	5	200	153.26	60	58.77	38
3	75	5	200	162.90	60	58.94	35
4	85	4	200	156.32	60	58.88	39
5	80	4	200	161.12	60	58.92	36
6	75	6	200	156.71	60	58.89	39
7	85	6	200	146.45	60	58.81	45
8	85	5	200	145.20	60	58.63	40
9	85	6	200	152.75	60	58.95	45
10	80	6	200	148.7	60	58.86	45
11	80	6	200	150.65	60	58.95	47
12	80	5	200	153.67	60	58.87	41
13	75	5	200	157.82	60	58.86	37

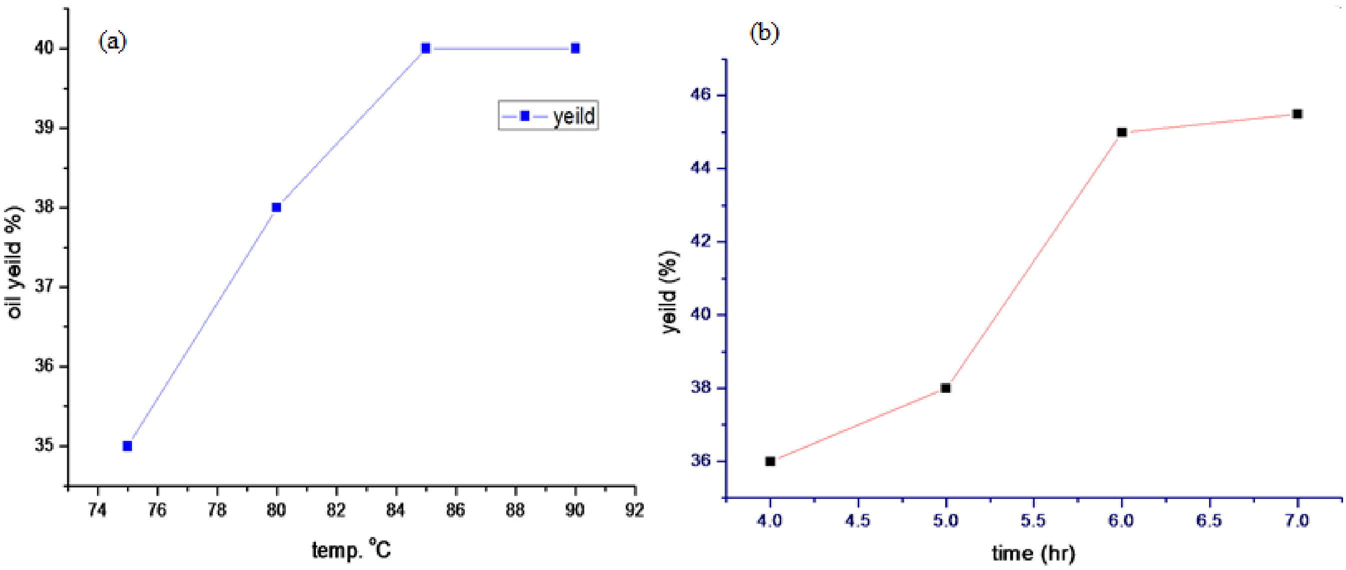


Fig.6. (a) Timperature and (b) time influences of oil yeil during oil extraction from blended algae with sludge

Table 3. Sequential model sum of squares

Source	Sum of Mean Squares	DF	Mean Square	F Value	Prob > F	
	20640.31	1	20640.31			
Linear	120.33	2	60.16	13.87	0.0013	Suggested
2FI	0.044	1	0.044	$9.180*10^{-3}$	0.9258	
Quadratic	20.31	2	10.15	3.09	0.1093	
Cubic	14.26	3	4.75	2.17	0.2338	Aliased
Residual	8.75	4	2.19			
Total	20804.00	13	1600.31			

Table 4. Lack of fit tests

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F	
Linear	34.61	6	5.77	2.64	0.1835	Suggested
2FI	34.57	5	6.91	3.16	0.1438	
Quadratic	14.26	3	4.75	2.17	0.2338	
Cubic	0.000	0				Aliased
Pure Error	8.75	4	2.19			

Table 5. Model summary statistics

Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	Press	
Linear	2.08	0.7351	0.6821	0.5298	76.97	Suggested
2FI	2.19	0.7354	0.6471	0.3817	101.21	
Quadratic	1.81	0.8594	0.7590	0.1692	135.99	
Cubic	1.48	0.9465	0.8396	+	Statistic not defined	Aliased

Table 6 is indicates that temperature ranges 75 to 85 ℃, time from 4 to 6 hrs and according to the factor ranges minimum oil yeild 35% and maximum oil yeild 47%. Using the design expert software the critical points of factors and maximum oil yeild are temperature 85 ℃, time 6hrs and desired oil yeild 45.45% selected.

Final equation model interms of actual factors is shown in equation (6).

Oil yeild, % = 0.46951*Temp + 3.70942*Time-16.71440 (6)

Equation (6) is maximum oil production model equation generated from experimental data analysis using design expert software, central composite method. So we can say this equation critical maximum formula with the range of temperature and time range. Graphical explanation of factor affect the temperature and time on the extraction of the lipid from blended waste sludge disposal and algae using centre of composite method in design expert software. Fig. 7 shows that plot of time, temperature with standard error of oil yield using design expert software central composite method.

Table 6. Constraints

Name Weight	Goal Importance	Lower Limit	Upper Limit
Temperature	is in range	75	85
Time	is in range	4	6
oil yeild	Maximize	35	47

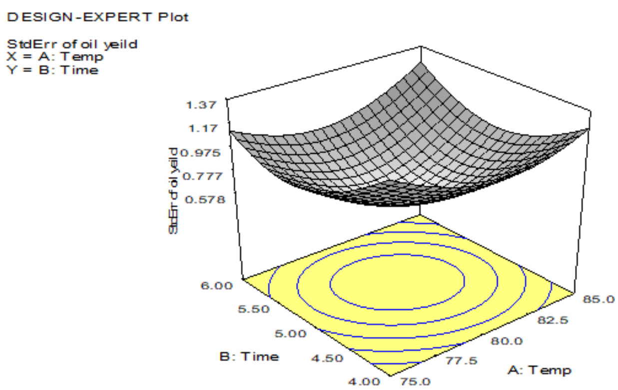


Fig. 7. Effect of temperature and time on oil extraction yield

Conclusions

In the present research it concluded that waste water treatment sludge disposal and algae lipid content are high and about equal in amount. In the extraction process by changing the factors (extraction time and extraction temperature) and constant solvent 200 ml and 20 g solute. In the laboratory experiments the repetitions where 13 times by changing the factors time and temperature. The critical maximum point of lipid extracted 47% at temperature 80℃ and for 6 hrs. This linear model of lipid extraction all most approaches to the critical optimum yield with the specified factor. The experimental results where analyzed and summarized by the design expert software. The lipid extraction and optimization where influenced by the major factor temperature and extract time, and critical optimum value of the factor where defined under this experimental result and from software analysis that depends on experimental results. Therefore this research becomes multi advantages in sustainability, if this research is applicable in industrial scale. In the developed country edible oil used for both biodiesel production and for supply feed. This co function of oil influences food security and increases raw material costs that leads the loss of product profitability. In the developing country like Ethiopia there was no capacity to use edible oil for biodiesel production in lack of economy and food security. But also there were increments of fuel engine consumers with unbalanced economy in developing country. Therefore waste material with high lipid contents like algae and waste water treatment sludge where the solution for such problem, by extracting oil (lipid) compositions for supplying biodiesel production. In this research it was waste recover and farther treatment for sludge (activated sludge), and extract the lipid (non-edible oil) from the blended sludge and algae for biodiesel production is the best alternative sustainable energy production and solves the problem of developing countries.

Conflicts of interest

Authors declare no conflict of interest.

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